

THE CLAIMS

What is claimed is:

- 5 1. A method of treating at least one bone or cartilage condition which comprises administering to an animal a therapeutically effective amount of an agent comprising creatine, or an analogue or pharmaceutically acceptable salt thereof, to treat bone or cartilage conditions.
- 10 2. The method of claim 1, wherein the animal is a mammal and the condition comprises a bone or cartilage disease, a bone fracture or defect, or a degenerative disease of cartilage.
- 15 3. The method of claim 2, wherein the mammal is a human and the disease comprises osteoporosis, osteoarthritis, or periodontitis.
4. The method of claim 2, wherein the mammal is a human and the agent is incorporated in a bone or cartilage graft that is applied to the bone fracture or defect.
- 20 5. The method of claim 4, wherein the agent is incorporated in at least one three dimensional construct of osteoblasts, chondrocytes, or mesenchymal stem cells designed for tissue engineering of the bone or cartilage condition and wherein the construct is administered to the bone or cartilage.
- 25 6. The method of claim 5, further comprising:
obtaining bone or cartilage forming cells from a healthy individual;
culturing the bone or cartilage forming cells in the presence of the agent to form a three-dimensional cell assembly; and
transferring the three-dimensional cell assembly to a specific location having
30 a bone or cartilage defect of the patient.
7. The method of claim 1, wherein the creatine, or analogue or pharmaceutically acceptable salt thereof, comprises creatine, creatine phosphate, creatine pyruvate, cyclocreatine, homocreatine, or homocyclocreatine.

8. The method of claim 1, further comprising administering at least one of: hormones, vitamins, growth factors, cytokines, matrix proteins, serum proteins, enzymes, calcium salts, fluoride salts, bone meal, hydroxyapatite, peptides, antioxidants, transferrin, selenium, boron, silicon, or nitric oxide.

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9. The method of claim 8, wherein, when administered, the hormones comprise parathyroid hormone-related protein, thyroid hormone, insulin, a sex steroid, prostaglandins, or glucocorticoids; the vitamins comprise 1,25(OH)₂ vitamin D₃ and analogues or metabolites of vitamin D, vitamin C/ascorbate, or retinoids; the growth factors comprise insulin-like growth factors (IGF), transforming growth factor b family (TGF-b), bone morphogenic proteins (BMP), basic fibroblastic growth factor (bFGF), platelet derived growth factor (PDGF), or epidermal growth factor (EGF); the cytokines comprise interleukins (IL), interferons, or leukaemia inhibitory factor (LIF); the matrix proteins comprise collagens, glycoproteins, hyaluronan, or proteoglycans; the serum proteins comprise albumin or alpha-2H5 glycoprotein; the enzymes comprise metalloproteinases, collagenases, gelatinases, stromelysins, plasminogen activators, cysteine proteinases, or aspartic proteinases; the fluoride salts comprise sodium fluoride or monosodium fluorophosphate; the peptides comprise amylin, vasoactive agents, or neuropeptides; the antioxidants comprise cysteine, N-acetyl-cysteine, glutathione, or vitamins A, C, D, or E.

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10. The method of claim 9, wherein, when matrix proteins are administered, the matrix proteins are glycoproteins comprising alkaline phosphatase, osteonectin (ON), gamma-carboxy glutamic acid-containing proteins, or arginine-glycine-asparagine-containing proteins, or proteoglycans comprising aggrecan, versican, biglycan, or decorin.

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11. The method of claim 9, wherein the hormone is parathyroid hormone and the parathyroid hormone is administered intermittently with the agent.

12. The method of claim 11, further comprising administering 1,25(OH)₂ 30 vitamin D₃ and analogues or metabolites of vitamin D, calcitonine, estrogen, or bisphosphonates to the individual.

13. The method of claim 1, wherein the bone comprises cells comprising 35 osteoblasts, periosteal cell, stromal bone marrow cells, satellite cells of muscle tissue, or mesenchymal stem cells, or a combination thereof.

14. The method of claim 1, wherein the cartilage comprises cells comprising chondroblasts or mesenchymal stem cells.

15. The method of claim 5, wherein the stem cells are cultured as monolayers, micromass cultures, or in a three-dimensional biodegradable scaffold.

16. The method of claim 6, wherein the three-dimensional cell assembly has a structure of a seeded sponge, foam, or membrane.

10 17. The method of claim 6, wherein 10 to 20 mM of creatine is concentrated in a culture medium containing one of 0.1% to 5% fetal calf serum or 10 to 250 μ g of ascorbic acid or an equivalent amount of a pharmaceutically acceptable ascorbate.

15 18. The method of claim 6, wherein a cell culture is started with 2,000 to 100,000 cells.

19. The method of claim 1, wherein the agent is essentially free of one or more of dihydrotriazine; dicyano-diamide; or creatinine.

20 20. The method of claim 1, wherein the agent is administered to a human patient in an amount of 1.4 to 285 mg per day.

21. The method of claim 1, wherein the creatine analogue has the general formula:

25 $Z_1 - \text{C}(\text{---}Z_2) - \text{---X-A-Y}$

and pharmaceutically acceptable salts thereof, wherein:

Y is selected from: $-\text{CO}_2\text{H}$, $-\text{NI-OH}$, $-\text{NO}_2$, $-\text{SO}_3\text{H}$, $-\text{C}(=\text{O})\text{NHSO}_2\text{J}$, and $-\text{P}(=\text{O})(\text{OH})(\text{OJ})$, wherein J is selected from: hydrogen, $\text{C}_1\text{-C}_6$ straight chain alkyl, $\text{C}_3\text{-C}_6$ branched alkyl, $\text{C}_2\text{-C}_6$ straight alkenyl, $\text{C}_3\text{-C}_6$ branched alkenyl and aryl;

A is selected from: C, CH, $\text{C}_1\text{-C}_5$ alkyl, $\text{C}_2\text{-C}_5$ alkenyl, $\text{C}_2\text{-C}_5$ alkynyl, and $\text{C}_1\text{-C}_5$ alkoyl chain, each having 0-2 substituents which are selected independently from:

K, where K is selected from: $\text{C}_1\text{-C}_6$ straight alkyl, $\text{C}_2\text{-C}_6$ straight alkenyl, $\text{C}_1\text{-C}_6$ straight alkoyl, 3-6 branched alkyl, $\text{C}_3\text{-C}_6$ branched alkenyl, $\text{C}_4\text{-C}_6$ branched alkoyl, K 35 having 0-2 substituents independently selected from: bromo, chloro, epoxy and acetoxy;

an aryl group selected from: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from: -CH₂L and -COCH₂L, wherein L is independently selected from: bromo, chloro, epoxy and acetoxy; and

5 -NH-M, wherein M is selected from: hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoxy, C₃-C₄ branched alkyl, C₃-C₄ branched alkenyl, and C₄-C₆ branched alkyl;

X is selected from: NR₁, CHR₁, CR₁, O and S, wherein R₁ is selected from:

hydrogen,

10 K where K is defined above; and

an aryl group selected from: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from: -CH₂L and -COCH₂L where L is defined above;

15 a C₅-C₉ Alpha-amino-omega-methyl-omega-adenosyl carboxylic acid attached via the omega-methyl carbon;

a C₅-C₉ Alpha-amino-omega-aza-omega-methyl-omega-adenosylcarboxylic acid attached via the omega-methyl carbon; and

a C₅-C₉ Alpha-amino-omega-thia-omega-methyl-omegaadenosylcarboxylic acid wherein A and X are connected by a single or double bond;

20 Z₁ and Z₂ are chosen independently from: =O, -NHR₂, -CH₂R₂, -NR₂OH; wherein, Z₁ and Z₂ may not both be =O and wherein R₂ is selected from:

hydrogen;

K, where K is defined above;

an aryl group selected from: a 1-2 ring carbocycle and a 1-2 ring heterocycle,

25 wherein the aryl group contains 0-2 substituents independently selected from: -CH₂L and -COCH₂L where L is as defined above;

a C₄-C₈ Alpha-amino-carboxylic acid attached via the omega - carbon;

B, wherein B is selected from: -CO₂H, -NHOH, NO₂, -SO₃H, -C(=O)NSO₂J and -P(=O)(OH)(OJ), wherein J is as defined above;

30 D-E, wherein D is selected from: C₁-C₃ straight chain alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoxy, and aryl; and E is selected from: -(PO₃)_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)_m-Q, wherein m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)_m-Q, where m is 0-3 and Q is a ribonucleoside

connected via the ribose of the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoxy, C₃-C₆ branched alky, C₁-C₆ branched alkenyl, C₄-C₆ branched alkoxy;

5 wherein E may be attached at any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

E, wherein E is as defined above,

provided that:

when E is aryl, B may be connected by an amide linkage;

10 if R₁ and at least one R₂ group are present, R₁ may be connected by a single or double bond to an R₂ group to form a cycle of 5 to 7 members;

if two R₂ groups are present, they may be connected by a single or double bond to form a cycle of 5 to 7 members; and

if R₁ is present and or Z₂ is selected from -NHR₂, -CH₂R₂ and -NR₂OH, then R₁ may

15 be connected by a single or double bond to the carbon or nitrogen of either Z₁ or to form a cycle of 4 to 7 members.

22. A method of promoting growth and mineralization of bone or cartilage cells and tissues which comprises administering to a subject in need of such treatment a

20 therapeutically effective amount of an agent comprising creatine, or an analogue or pharmaceutically acceptable salt thereof, to promote growth and mineralization of bone or cartilage therein.

23. A method of improving acceptance and osseous integration of bone

25 implants which comprises administering to a subject in need of such treatment a therapeutically effective amount of an agent comprising creatine, or an analogue or pharmaceutically acceptable salt thereof, to improve acceptance and osseous integration of bone implants.

30 24. A method for accelerating healing in a subject having a defect in bone or cartilage tissue caused by trauma, surgery, or a degenerative disease, which method comprises administering to the subject a therapeutically effective amount of a creatine compound, analogue or pharmaceutically acceptable salt thereof, or a creatine kinase.

25. A composition useful for the treatment of defects in bone or cartilage tissue of animals or humans caused by trauma or surgery, the composition comprising a creatine compound, analogue or pharmaceutically acceptable salt thereof, the composition being suitable for oral administration and including a pharmacologically suitable carrier to
5 improve bioavailability.

26. The composition of claim 25, wherein the carrier is selected from: carbohydrates, maltodextrins, and dextrose.

10 27. A method of preparing an agent for treatment of bone or cartilage cells or tissues, comprising:

removing bone or cartilage forming cells from a healthy subject;

adding the bone or cartilage forming cells to a cell culture;

transfected the bone or cartilage forming cells with complementary DNA

15 coding for creatine kinase isoforms and made to overexpress creatine kinase isoenzyme(s); and

expanding and cultivating the bone or cartilage forming cells to form *in vitro* genetically engineered cartilage or bone tissues transplantable into areas of cartilage or bone defects of the healthy subject.

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